

AD-A082 132

CINCINNATI UNIV OH COLL OF MEDICINE F/6 6/15
EXTRACTION OF LABELLED WR-158,122 FROM RAT AND MONKEY URINE AND--ETC(U)
FEB 80 C C SMITH, S F MATTINGLY, G F WOLFE DAMD17-79-C-9106

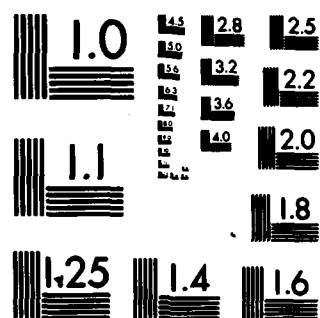
UNCLASSIFIED

NL

1-1
8-1-80



END
DATE
FILMED
4-80
DTIC



MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A

AD A082132

Unclassified

LEVEL II

AD _____

(9) Interim Report No. 3, 1

Extraction of Labelled WR-158,122 from Rat and
Monkey Urine and Bile. Initial Studies

Carl C. Smith, Ph.D.
Steele F. Mattingly, D.V.M.

Geraldine F. Wolfe, M.S.
David H. Bauman, D.V.M.
Gary L. Keller, D.V.M.

February 13, 1980

Revised February 28, 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-79-C-9106

Department of Environmental Health and
Department of Laboratory Animal Medicine
University of Cincinnati College of Medicine
Cincinnati, Ohio 45267

DDC Distribution Statement

Approved for public release; distribution unlimited.
The findings in this report are not to be construed
as an official Department of the Army position unless
so designated by other authorized documents.

Unclassified

80

3

7

059

DDC FILE COPY

083460

DTIC
SELECTED
MAR 19 1980

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Interim Report No. 3	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Extraction of Labelled WR-158,122 from Rat and Monkey Urine and Bile. Initial Studies		5. TYPE OF REPORT & PERIOD COVERED Interim Report No. 3. February 13, 1980
7. AUTHOR(s) Carl C. Smith, Ph.D.		6. PERFORMING ORG. REPORT NUMBER Interim Report No.3
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Environmental Health and Department of Laboratory Animal Medicine Univ. of Cinti. Col. of Med., Cinti, OH 45267		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-79-C-9106
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Developmental Command Fort Detrick, Frederick, Maryland 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 2192040 975-8119 612770.80300 2583 S18064
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE February 13, 1980 Revised February 28, 1980
		13. NUMBER OF PAGES 16
		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited. The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) sulfonylquinazoline WR-158,122 2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline rhesus monkey bile duct cannulated rat bile duct ligated rat organic solvent extraction urine extractions bile extractions labelled drug		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Urine and bile samples from control (untreated) rats and rhesus monkeys as well as from bile duct ligated (BiDuLi) rats and bile duct cannulated (BiDuCa) rats and monkeys treated with WR-158,122- ¹⁴ C have been extracted with a series of organic solvents in an attempt to define the profile of biliary and urinary metabolites. Extraction of a urine sample from a BiDuCa monkey demonstrated that when the urine was saturated with KBr very polar solvents such as n-propanol (NPRO), pyridine, dimethylformamide or methanol formed one phase (with some salt) and the solvent phase contained all or nearly all of the ¹⁴ C		

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

(Block 20 continued)

activity. Solvents forming 2 phases such as ethylene dichloride: 2-ethyl-hexanol (8:2, EDC-2EH), ethyl acetate, acetonitrile (ACN) or tetrahydrofuran (THF) extracted increasing amounts of ^{14}C -containing metabolites ranging from 8.3% to 66.2%. NPRO, EDC-2EH, ACN and THF extracted all or nearly all of the ^{14}C activity from control simian urine and bile and from control rat urine. EDC-2EH, ACN and THF extracted increasing amounts of ^{14}C from a 72 hr monkey treatment bile but even THF extracted only 27% of the total ^{14}C content. The total parent drug in this sample was 3% or less. The ^{14}C activity in a 12 hr treatment bile sample from a BiDuCa rat gave an extraction profile not much different from that obtained with simian treatment bile; less than 4% of the activity comprised parent drug. When one compares 24 hr urine samples from BiDuCa and BiDuLi rats one finds that the only significant difference in the profiles is in the amount of ^{14}C activity extracted by EDC-2EH which was about 14% for the BiDuLi rat and 23% for the BiDuCa animal. These data provide almost indisputable evidence that WR-158,122 is absorbed and metabolized in the albino rat and rhesus monkey and the proportion of extractable ^{14}C activity is greater in the urine than in the bile. This is especially true for the monkey.

Accession For	
NTIS GRA&I	☒
DDC TAB	☐
Unannounced	☐
Justification	
By _____	
Dist. 10/10/10/	
Available for _____	
Dist	Available and/or special
A	

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

TABLE OF CONTENTS

	<u>Page</u>
DD Form 1473	
List of Tables	iii
I. Introduction	1
II. Materials and Methods	1
III. Results	2
IV. Discussion	5
V. Summary	6
VI. Tables	8-12
VII. Appendix	13
VIII. Signature Page	15
IX. Distribution List	16

List of Tables

<u>Table</u>		<u>Page</u>
1	Extraction of WR-158,122 ¹⁴ C Treatment Urine from a Bile Duct Cannulated Rhesus Monkey Single Oral Dose 5 mg/kg	8
2	Extraction of Control Monkey Urine Spiked with WR-158,122 ¹⁴ C	9
3	Extraction of WR-158,122 Treatment Bile from a Bile Duct Cannulated Monkey Single Oral Dose 5 mg/kg	9
4	Extraction of Control Monkey Bile Spiked with WR-158,122 ¹⁴ C	10
5	Extraction of WR-158,122 Treatment Bile from a Bile Duct Cannulated Rat Single Oral Dose 10 mg/kg	10
6	Extraction of WR-158,122 ¹⁴ C Treatment Urine from a Bile Duct Ligated Rat Single Oral Dose 10 mg/kg	11
7	Extraction of WR-158,122 Treatment Urine from a Bile Duct Cannulated Rat Single Oral Dose 10 mg/kg	11
8	Extraction of Control Rat Urine Spiked with WR-158,122 ¹⁴ C	12

Interim Report No. 3

In our first interim report (11/20/79) we described the data we obtained on the absorption, excretion and distribution of ^{14}C following single oral doses of WR-158,122- ^{14}C in control, bile duct ligated (BiDuLi) and bile duct cannulated (BiDuCa) rats. The second report (1/18/80) summarized data on blood levels and excretion of ^{14}C derived from WR-158,122 in a BiDuCa monkey following single oral doses. In this report we are presenting our initial findings in developing extraction procedures for separating and isolating WR-158,122 and its metabolites from urine and bile using the samples obtained in the course of our previous studies.

The general approach consists of extracting biological samples with a series of solvents with varying solubility parameters (see appendix). Because some of the desirable solvents are miscible with water, a salting out procedure was used to provide, whenever possible, separate solvent and aqueous phases.

MATERIALS AND METHODS

These initial studies were carried out on urine and bile from control (untreated) rats and monkeys and treatment urine and/or bile from BiDuLi and BiDuCa rats and a BiDuCa monkey.

The ^{14}C standard used to spike control monkey and rat urine and monkey bile was prepared as follows. One ml of the treatment solution used in Interim Report No. 2 was diluted to 10 ml with DMSO. One ml of this standard was diluted to 25 ml with control urine or bile giving spiked samples which contained about 17,000 dpm of WR-158,122- ^{14}C per ml.

The general protocol used in these initial extraction studies is described, for convenience, at the bottom of the first table and was the same in all the experiments. We have concentrated our attention on organic solvents that

form two phases after the addition of KBr, a neutral salt, to the urine or bile. The procedure appears to work well and so far has not been plagued by formation of emulsions.

RESULTS

In Table 1 one observes that all or almost all the ^{14}C in the urine was present in the solvent phase in those solvents (n-propanol, dimethylformamide, pyridine and methanol) which formed only one phase (and salt).

However, solubility of parent drug and/or ^{14}C -containing metabolites in the solvents forming 2 phases was quite variable. EDC-2-ethyl hexanol (8:2) (EDC-2EH), a solvent mixture more polar than EDC (alone) appeared to remove only about 8.3% of the total urinary ^{14}C . This fraction probably consists of parent drug and possibly a relatively nonpolar metabolite(s). More polar solvents such as ethyl acetate, acetonitrile (ACN) or tetrahydrofuran (THF) extracted increasing quantities of urinary metabolites.

The results in Table 2 show that one can quantitatively extract the parent compound from spiked control monkey urine with 3 of the two phase solvents and with a representative single phase solvent, n-propanol. Therefore, we would conclude that all of the parent drug in monkey treatment urine was extracted by all of the organic solvents shown in Table 1. In addition, there were increasing amounts of metabolites extracted by ACN and THF; the quantity extracted depending on the polarity of the solvent used. We assumed that all single phase solvents would function alike; therefore, after the first experiment we used only one single phase solvent, n-propanol. From its solubility parameter it should be the least polar of the single-phase solvents.

Application of the same extraction scheme to a monkey treatment bile

sample provided the results shown in Table 3. If all the parent drug is extracted by EDC-2EH, as one would assume (see Table 4), then one must conclude that the amount of parent compound in this animal's bile at 72 hr is no more than about 3% of the total ^{14}C . Also the ^{14}C -containing metabolites in this sample must be quite polar since even THF extracted only about 27% of the total ^{14}C in the bile.

The data in Table 4 indicate that WR-158,122- ^{14}C is quantitatively extracted from spiked control monkey bile. These results are essentially parallel to those in Table 2.

When the extraction procedure was applied to a 12 hr bile sample from a BiDuCa rat dosed with WR-158,122- ^{14}C we obtained the results shown in Table 5. The EDC-2EH soluble fraction was 3.9%, ACN and THF extracted 14 and 17% respectively and as would be expected almost all of the ^{14}C was soluble in N-propanol.

When urine from a representative BiDuLi rat was extracted in the same fashion (Table 6) one notes that more ^{14}C was extracted by these solvents from the urine than from the bile, suggesting either that lesser amounts of extractable metabolites are present in bile or that something in bile interferes with the transfer of ^{14}C -containing metabolites to the organic phases. This will be taken up in the discussion.

Urine from a rat with BiDuCa and treated with an oral dose of WR-158,122- ^{14}C gave an extraction profile (see Table 7) which was similar to that in Table 6 except for one point. EDC-2EH extracted more ^{14}C after BiDuCa and this is opposite from what we would have expected. However, since there was less ^{14}C excreted in the urine in BiDuCa rats perhaps a higher percentage may be

"parent-like" compounds (extractable with EDC-2EH).

Finally, control rat urine spiked with WR-158,122-¹⁴C gave the same results as were obtained with control monkey urine and bile indicating that WR-158,122 was completely extractable from urine or bile by any of the solvents we used.

DISCUSSION

These data suggest strongly that one can develop an extraction procedure for characterizing the metabolites of WR-158,122 in either bile or urine samples from both rats and rhesus monkeys. The solvents can be applied sequentially and thus would permit us, hopefully, to evolve a scheme for isolating individual metabolite fractions. These fractions can be further defined using TLC or HPLC and such experiments are now being planned.

The low recovery of ^{14}C activity from bile may be the result of absolute differences in amounts of WR-158,122 and metabolites present or may reflect an undefined inhibiting effect of bile constituents on extraction. This ambiguity will be resolved in our next interim report.

Several other preliminary extraction studies are also underway in which we will saturate urine or bile with an acidic salt such as $(\text{NH}_4)_2\text{SO}_4$ or basic salts such as Kacetate (pH 9.7) or K_2CO_3 (pH 11.7). It will be interesting to compare these results with those obtained with KBr. We also need to explore more non-polar solvents so that we can improve, if possible, the specificity of the initial extraction for parent drug.

SUMMARY

1. Urine and bile samples from control (untreated) rats and rhesus monkeys as well as from bile duct ligated (BiDuLi) rats and bile duct cannulated (BiDuCa) rats and monkeys treated with WR-158,122- ^{14}C have been extracted with a series of organic solvents in an attempt to define the profile of biliary and urinary metabolites.

2. Extraction of a urine sample from a BiDuCa monkey demonstrated that when the urine was saturated with KBr very polar solvents such as n-propanol (NPRO), pyridine, dimethylformamide or methanol formed one phase (with some salt) and the solvent phase contained all or nearly all of the ^{14}C activity. Solvents forming 2 phases such as ethylene dichloride: 2-ethylhexanol (8:2, EDC-2EH), ethyl acetate, acetonitrile (ACN) or tetrahydrofuran (THF) extracted increasing amounts of ^{14}C -containing metabolites ranging from 8.3% to 66.2%.

3. NPRO, EDC-2EH, ACN and THF extracted all or nearly all of the ^{14}C activity from control simian urine and bile and from control rat urine.

4. EDC-2EH, ACN and THF extracted increasing amounts of ^{14}C from a 72 hr monkey treatment bile but even THF extracted only 27% of the total ^{14}C content. The total parent drug in this sample was 3% or less.

5. The ^{14}C activity in a 12 hr treatment bile sample from a BiDuCa rat gave an extraction profile not much different from that obtained with simian treatment bile; less than 4% of the activity comprised parent drug.

6. When one compares 24 hr urine samples from BiDuCa and BiDuLi rats one finds that the only significant difference in the profiles is in the amount of ^{14}C activity extracted by EDC-2EH which was about 14% for the BiDuLi rat and 23% for the BiDuCa animal.

7. These data provide almost indisputable evidence that WR-158,122 is absorbed and metabolized in the albino rat and rhesus monkey and the proportion of extractable ¹⁴C activity is greater in the urine than in the bile. This is especially true for the monkey.

Table 1

Extraction of WR-158,122 ¹⁴C Treatment Urine* From
A Bile Duct Cannulated Rhesus Monkey

Single Oral Dose 5 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.2	2	8.3
Ethyl acetate	5.1	2	24.2
Acetonitrile	5.0	2	45.6
Tetrahydrofuran	4.9	2	66.2
N-propanol	5.9	1	93.7
Dimethylformamide	5.9	1	94.2
Pyridine	5.8	1	95.4
Methanol	6.0	1	96.5

*48 hr treatment urine containing 12,400 dpm/ml

Protocol:

1. Dissolve 0.5g KBr in each 1 ml aliquot in a graduated screw cap centrifuge tube.
2. Add 5 ml solvent.
3. Shake for 20 min and centrifuge.
4. Remove organic phase after recording color and volume of each phase and salt, if any.

Table 2
Extraction of Control Monkey Urine
Spiked with WR-158,122 ¹⁴C

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.1	2	98.9
Acetonitrile	5.4	2	99.8
Tetrahydrofuran	5.2	2	104.1
N-propanol	5.9	1	97.6

Protocol same as Table 1.

Table 3
Extraction of WR-158,122 Treatment Bile*
from a Bile Duct Cannulated Monkey
Single Oral Dose 5 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.1	2	3.0
Acetonitrile	5.1	2	18.6
Tetrahydrofuran	5.2	2	26.8
N-propanol	5.7	1	103.8

*72 hr treatment bile containing 15,500 dpm/ml

Protocol same as Table 1.

Table 4

Extraction of Control Monkey Bile
Spiked with WR-158,122 ¹⁴C

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.1	2	95.7
Acetonitrile	5.1	2	92.4
Tetrahydrofuran	5.1	2	95.2
N-propanol	6.1	1	97.3

Protocol same as Table 1.

Table 5

Extraction of WR-158,122 Treatment Bile*
from a Bile Duct Cannulated Rat
Single Oral Dose 10 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.05	2	3.9
Acetonitrile	5.2	2	14.4
Tetrahydrofuran	5.1	2	16.9
N-propanol	5.7	1	87.1

*12 hr treatment bile containing 62,000 dpm/ml.

Protocol same as Table 1.

Table 6

Extraction of WR-158,122 ¹⁴C Treatment Urine* From
A Bile Duct Ligated Rat
Single Oral Dose 10 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery(%)
EDC-2-Ethylhexanol(8:2)	5.3	2	13.7
Acetonitrile	5.2	2	30.9
Tetrahydrofuran	5.3	2	38.1
N-propanol	6.0	1	87.1

*24 hr treatment urine containing 27,100 dpm/ml.

Protocol same as Table 1.

Table 7

Extraction of WR-158,122 Treatment Urine*
from a Bile Duct Cannulated Rat
Single Oral Dose 10 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery(%)
EDC-2-Ethylhexanol(8:2)	5.0	2	23.3
Acetonitrile	5.2	2	30.2
Tetrahydrofuran	5.0	2	38.9
N-propanol	5.7	1	88.4

*24 hr treatment urine containing 13,200 dpm/ml.

Protocol same as Table 1.

Table 8

Extraction of Control Rat Urine
Spiked with WR-158,122 ^{14}C

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery(%)
EDC-2-Ethylhexanol	5.2	2	99.7
Acetonitrile	5.3	2	95.3
Tetrahydrofuran	5.2	2	103.0
N-propanol	5.9	1	98.4

Protocol same as Table 1.

APPENDIX

Common Solvents Listed According to Increasing Solubility Parameter (1)

Solvent	Sol. Par. (2,3)	H ₂ Bond- ing (2)	Boil. Point	S.G.	Sol. of H ₂ O in Solv. (%)	Mol. Weight	Dielec- tric Const. (4)	Dipole Debye μ (4)
n-pentane	7.0	low	36.1	0.62	0.01	72.15	1.844	0.00
n-hexane	7.3	low	68.7	0.66	0.01	86.17	1.890	0.08
diethyl ether	7.4	0.19	34.5	0.71	1.5	74.12	4.335	1.15
n-heptane	7.4	low	98.4	0.68	0.015	100.2	1.924	0
cyclohexane	8.2	low	80.7	0.78	0.01	84.16	2.023	0
methyl, n-hexyl ketone	8.4	med.	173.5	0.82	insol.	128.21	-	-
carbon tetra- chloride	8.6	low	76.8	1.58	0.01	153.84	2.238	0.00
diethyl ketone	8.8	med.	101.7	0.81	∞	86.13	17.00	2.70
toluene	8.9	low	110.6	0.86	0.06	92.13	2.379	0.39
ethyl acetate	9.1	0.12	77.0	0.90	3.3	88.10	6.02	1.81
benzene	9.2	low	80.1	0.87	0.05	78.11	2.284	0
chloroform	9.3	low	61.1	1.48	0.07	119.39	4.806	1.15
methyl ethyl ketone	9.3	med.	79.5	0.80	87.4	72.10	18.51	2.747
chlorobenzene	9.5	0.02	131.7	1.10	0.05	112.56	5.621	1.56
ethylene di- chloride	9.8	low	83.5	1.25	0.15	98.97	10.36	2.06
p-dioxane	9.9	0.14	101.3	1.02	∞	88.10	2.209	0.45
acetone	10.0	0.14	56.2	0.79	∞	58.09	20.70	2.72
isooxyl alcohol	10.0	high	132.0	0.80	2.67	88.15	14.7	1.82
tert.-butyl alcohol	10.6	high	82.4	0.78	∞	74.12	10.9	1.66
pyridine	10.7	0.27	115.3	0.98	∞	79.1	12.3	2.20
sec. butyl al- cohol	10.8	high	99.5	0.80	ca 12	74.12	15.8	-

Solvent	Sol. Par. (2,3)	H ₂ Bonding (2)	Boil. Point	S.G.	Sol. of H ₂ O in Solv. (%)	Mol. Weight	Dielectric Const. (4)	Dipole Debye μ (4)
n-amyl alcohol	10.9	high	138.1	0.81	2.19	88.15	13.9	1.8
nitroethane	11.1	low	114	1.04	0.9	75.07	28.06	3.19
n-butyl alcohol	11.4	high	117.7	0.81	20.5	74.12	17.1	1.68
isopropyl alcohol	11.5	high	82.4	0.78	∞	60.09	18.3	1.68 (v)
acetonitrile	11.9	0.09	81.6	0.78	∞	41.05	37.5	3.37
n-propyl alcohol	11.9	high	97.2	0.80	∞	60.09	20.1	1.657
benzyl alcohol	12.1	high	205.5	1.04	ca 4	108.13	13.1	1.66
furfuryl alcohol	12.5	high	170	1.13	∞ (unstable)	98.1	-	1.92
ethyl alcohol	12.7	high	78.3	0.79	∞	46.09	24.30	1.68 (v)
methyl alcohol	14.5	0.28	64.5	0.79	∞	32.04	32.68	1.664
formamide	> 16.1	high	210.5	1.13	∞	45.04	109.5	3.37
glycerol	16.5	high	290	1.26	∞	92.09	42.5	2.56
water	23.4	high	100	1.0	-	18.02		

1. Solubility parameter $\delta = (\Delta E/v)^{1/2}$ where ΔE is the energy of vaporization to a gas at zero pressure and v is the molal volume of the liquid ($v = \text{mol. wt.} / \text{density}$).
2. Burrell, H. Solubility Parameters, Interchemical Review, 14:3 (1955); low = < 0.08, high = 0.15 or >.
3. Hildebrand, J. and Scott, R. "The Solubility of Nonelectrolytes," New York: Reinhold Pub. Corp. 1950.
4. Weissberger, A. "Technique of Organic Chemistry" Vol VII, New York: Interscience Publishers.

SIGNATURE PAGE



Carl C. Smith, Ph.D.

2/13/80

Date

Revised 2/28/80

Distribution List

12 copies	Director (ATTN: SGRD-UWZ-AG) Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D.C. 20012
4 copies	HQDA (SGRD-SI) Fort Detrick Frederick, MD 21701
2 copies	Defense Documentation Center ATTN: DDC-DCA Cameron Station Alexandria, Virginia 22314
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 copy	Superintendent Academy of Health Sciences, U.S. Army ATTN: AHS-COM Fort Sam Houston, Texas 78234